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Temporal and Spatial Distribution of Ferredoxin Isoproteins in Tomato Fruit[1]

Koh Aoki and Keishiro Wada*

Department of Biology, Faculty of Science, Kanazawa University, Kakuma, Kanazawa 920–11, Japan

Five ferredoxin (Fd) isoproteins (FdA, FdB, FdC, FdD, and FdE) were isolated from tomato (*Lycopersicon esculentum* cv Momotaro) fruit. These isoproteins showed differential temporal and spatial accumulation patterns. FdA and FdC were present in leaves, FdE was present in roots, and FdB and FdD were fruit-specific. During fruit growth, the relative abundance of FdA decreased and that of FdE increased. The FdE/FdA ratio was higher in the inner tissues of the fruit than in the outer tissue, and it was correlated with starch accumulation. In dark-grown fruit the contents of FdA, FdB, and FdC, as well as chlorophyll, decreased remarkably relative to their light-grown counterparts; however, the contents of FdE and starch did not change significantly. Under in vitro conditions FdE showed higher cytochrome *c* reduction activity than FdA and FdB. These results, together with their N-terminal sequences, indicate that both photosynthetic- and heterotrophic-type Fd isoproteins are present in tomato fruit.

Fd is an acidic, small plastid protein that has an iron-sulfur cluster as a redox center. In higher plants Fd was originally found to participate in the photoreduction of $NADP^+$ by chloroplasts (Arnon, 1965). Later, it was found to be present in nonphotosynthetic organs such as roots (Wada et al., 1986) and etiolated seedlings (Kimata and Hase, 1989), and then to mediate electron transfer from NADPH to Fd-dependent enzymes (e.g. nitrite reductase, sulfite reductase, and Fd-glutamate synthase) in a light-independent manner (Buchanan, 1980; Suzuki et al., 1985). Studies on leaf and root Fds indicate considerable differences in their amino acid sequences and chemical properties (Wada et al., 1989; Hase et al., 1991b).

Accumulation of Fd isoprotein is primarily controlled at the transcriptional level, and accumulation of mRNAs of leaf Fd isoproteins is regulated by light (Kaufman et al., 1986; Sagar et al., 1988; Hase et al., 1991a; Casper and Quail, 1993). Genomic clones encoding leaf Fd isoproteins have been obtained from pea (Elliott et al., 1989), *Arabidopsis thaliana* (Somers et al., 1990), and wheat (Bringloe et al., 1995). Studies using transgenic tobacco have demonstrated that sequences in both upstream and downstream regions of the transcription initiation site are required for light-regulated expression of pea *Fed-1* and Arabidopsis *fedA* (Gallo-Meagher et al., 1992; Bovy et al., 1995). cDNAs of root Fd isoproteins have been cloned from maize (Hase et al., 1991a) and rice (Doyama et al., 1994). Accumulation of mRNA of maize root Fd is regulated independently of light (Hase et al., 1991a), and nitrate-inducible accumulation of mature Fd protein in pea roots also has been reported (Bowsher et al., 1993). Following the transcription, Fd is synthesized as a large precursor and is imported to the plastid stroma after posttranslational processing. It has been reported that the photosynthetic activity of plastids induces a differential import efficiency of leaf and root Fd precursors of maize (Hirohashi et al., 1994). These differences in light requirement for transcription and import imply that photosynthetic properties of the plastids and parent tissues may affect the distribution of Fd in the plant.

There are many cases in which nonphotosynthetic plant tissues become photosynthetic and vice versa. One of the best-known examples is the greening of etiolated seedlings in the light. Kimata and Hase (1989) investigated a temporal change in the accumulation of four Fd isoproteins in maize (*Zea mays* L.) seedlings during greening. They reported that two Fd isoproteins were light-inducible and detected only in leaves, whereas the others showed a constitutive accumulation in all parts of the seedling.

Another well-known example is fruit ripening, during which chloroplasts are typically converted to carotenoid-containing chromoplasts. We have previously reported the purification of Fd isoproteins from tomato fruit (*Lycopersicon esculentum*) and their accumulation at different developmental stages (Green et al., 1991; Kamide et al., 1995). FdA and FdC were found in leaves, whereas FdB was found only in fruit. During ripening the relative abundance of FdA and FdC apparently decreased and that of FdB seemed to increase.

In the present study we carried out a more detailed study of Fd isoproteins in tomato fruit and identified a novel isoform in addition to the isoforms found previously. The accumulation of Fd isoproteins was investigated at different developmental stages and in different tissues of the fruit. Additionally, Chl, lycopene, and starch were quantified as markers of chloroplasts, chromoplasts, and starch-containing plastids, respectively. We also focused our attention on the comparison of photosynthetic and heterotrophic tissue rather than on chloroplast- and chromoplast-containing tissue.

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* Corresponding author; e-mail keiwada@kenroku.ipc.kanazawa-u.ac.jp; fax 81–762–64–5745.

Abbreviations: B, breaker; Chl, chlorophyll; DAA, days after anthesis; FNR, Fd-$NADP^+$ oxidoreductase; IMG, immature green; MG, mature green; OPP, oxidative pentose-P pathway; RR, red ripe; T, turning.

MATERIALS AND METHODS

Ripe tomato (*Lycopersicon esculentum* cv Momotaro) fruit for Fd purification was purchased from a local market. For other experiments, tomato fruit was harvested from plants grown in a greenhouse from August to November, 1995, under natural sunlight. Using the Hyponica system (Kyowa, Osaka, Japan) in a greenhouse under natural conditions, leaf and root tissues were obtained from tomato plants grown hydroponically. Flowers were tagged on the days of anthesis, and the fruit was immediately subjected to protein or pigment extraction.

The stage when the fruit is not fully expanded and its locule gels are not formed is referred to as the IMG stage. At the MG stage, fruit is fully expanded and its locule gels have developed; at the B stage, the first visible red sign is found at the bottom of the fruit; at the T stage, the red area is spreading; and at the RR stage, fruit is red over the entire surface.

The peeled outer skin of the fruit was used as the exocarp after the remaining pericarp portion was removed. The remainder was cut apart from the base of the radial pericarp after removal of the locular tissue, and the outer and the inner parts were used as the inner pericarp and the columella, respectively.

Growing Tomato Fruit in the Dark

Tomato fruit was grown in the dark essentially as described by Cheung et al. (1993). The fruit was wrapped in two layers of paper bags with black linings soon after formation. Plants were allowed to grow normally. Fruit ripened in the dark was collected about 1 week after the harvest of light-grown RR fruit.

Extraction, Purification, and Assay of Fd

Fd extraction and purification were carried out according to the conventional method (Wada et al., 1989). The eluate from the first DEAE-cellulose column was fractionated by adding ammonium sulfate to 70% saturation, and the supernatant solution was concentrated with a small Butyl-Toyopearl column (Tosoh, Tokyo) (Matsubara and Wada, 1988). The resulting crude Fd preparation was used either for electrophoresis or for further purification.

Fd was assayed by the method described by Morigasaki et al. (1990). An extinction coefficient of 19.1 mM^{-1} cm^{-1} at 550 nm was used to calculate Cyt *c* reduction rates. For saturation-curve determination, 20 nM FNR purified from tomato fruit at the RR stage was used, and Fd concentration was determined using an extinction coefficient of 10.4 mM^{-1} cm^{-1} at 422 nm (Wada et al., 1974).

Electrophoresis and Immunoblotting

Protein concentration was determined by the method of Bradford (1976). PAGE was carried out without SDS using a 15% gel concentration at 16 mA for 2.5 h, according to the method of Williams and Reisfeld (1964). Fd was blotted onto a PVDF membrane essentially by the method of Kimata and Hase (1989). Transfer buffer was as described by Hirano and Watanabe (1990) with some modification, which included the lowering of methanol concentration to 10% and the removal of SDS, except for buffer C (25 mM Tris base, 40 mM ϵ-amino-*n*-caproic acid, 10% methanol, and 0.04% SDS, pH 9.5), to increase the transfer efficiency. Before blotting, polyacrylamide gels were warmed at 60°C for 10 min in transfer buffer C without SDS. Antisera against tomato-fruit Fd isoproteins were prepared by Bio-Chiba (Watsuka, Kyoto, Japan), using 1 mg of FdA and FdE. Fd (0.25 mg) emulsified with Freund's complete adjuvant was injected four times into a rabbit at weekly intervals. Visualization of immunoblotting was carried out using the enhanced chemoluminescence detection system (Amersham) according to the manufacturer's instructions.

Protein Sequencing and Image Analysis

N-terminal amino acid sequencing was performed using a protein sequencer (model 476A, Applied Biosystems). Densitometry of Coomassie brilliant blue-stained gels or immunoblotting films was performed using BIO-PROFIL 1-D (Vilber Lourmat, Marne La Vallée, France).

Quantification of Pigments and Starch

Fresh tissue was homogenized in cold acetone. Chl was determined in 80% (w/v) acetone extract by the method of Arnon (1949).

For lycopene determination, pigments were extracted from acetone extract by partitioning against diethyl ether. The diethyl ether fraction was saponified by adding the same volume of 10% (w/v) KOH in methanol for 1 h, followed by partitioning against 5% (w/v) NaCl in water. Carotenoids were separated by HPLC on a C_{18} column (1.5 mm × 250 mm, Capcell Pak UG120, Shiseido, Tokyo, Japan) using an elution solvent of acetonitrile:methanol:dichloromethane:hexane (85:10:2.5:2.5, v/v) at a flow rate of 200 μL/min, and monitored at 450 nm. Lycopene content was determined by integration of the peak area. Commercial lycopene (Sigma) was used as a standard.

Starch was extracted and quantified by the procedure of Wang et al. (1993), which was modified to include additional purification by passage through a Percoll (Pharmacia) gradient (Delrue et al., 1992).

RESULTS

Purification of Fd Isoproteins from Tomato Fruit at the RR Stage

Fd isoproteins were separated on a mono-Q HR5/5 column following DEAE-cellulose and Butyl-Toyopearl chromatography. Four peaks showing Fd activity were eluted at different NaCl concentrations (Fig. 1). In the subsequent native PAGE, peaks A, B, and E migrated as a single band and were designated FdA, FdB, and FdE, respectively, whereas peak C split into two bands (Fig. 2). A result of the analysis of the N-terminal amino acid sequence (Fig. 3) confirmed that both bands were distinctive Fd isoproteins; the upper and lower bands were designated FdC and FdD, respectively.

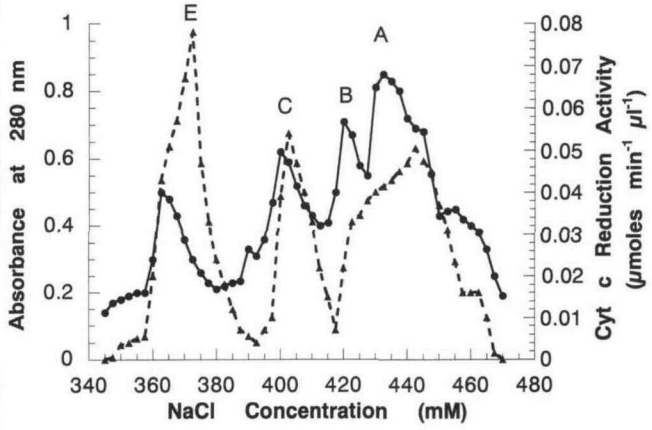

Figure 1. Elution profile of Fd isoproteins on Mono-Q HR 5/5 fast protein liquid chromatography. A, B, C, and E indicate activity peaks corresponding to FdA, FdB, FdC, and FdE, respectively. ●, A_{280}; ▲, Cyt *c* reduction activity per μL of each fraction.

Analysis of the N-terminal amino acid sequences shows that FdD and FdE are novel forms of Fd, and FdA, FdB, and FdC are identical to the forms previously purified (Kamide et al., 1995). FdE had Asn in the N-terminal position, although most of plant Fds have Ala or Ser in this position. This is the first example, to our knowledge, of plant Fds that have Asn in the N terminus, except for Fd from *Peridinium bipes* (Dinophyceae) in which the N-terminal amino acid is Phe because of the lack of some N-terminal residues (Uchida et al., 1988). FdD and FdE sequences are similar to those of root Fds, having amino acids identical to the root Fds of radish (Wada et al., 1989) and spinach (Morigasaki et al., 1990) in the 2nd, 11th, and 14th positions. In addition, FdE has His in the 15th position, as observed in maize root Fd (Hase et al., 1991a). On the other hand, FdA and FdC are similar to each other, having 77% homology in the N-terminal region. An FdB sequence also shows high similarity to those of leaf-type Fd isoproteins, because it has Thr in the 2nd position, Ile-Thr in the 8th and 9th positions, and Ser in the 11th position, as found in most of the leaf Fds.

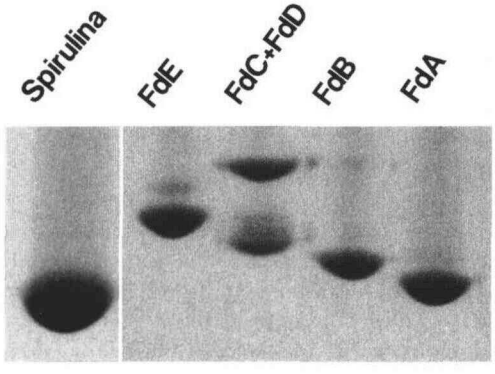

Figure 2. Native PAGE of purified Fd isoproteins. Spirulina Fd was used as a migration marker. Five milligrams of purified isoprotein was applied to each lane. In the FdC+FdD lane, the upper band is FdC and the lower band is FdD.

Temporal Accumulation Pattern of Fd Isoproteins during Tomato Fruit Development

For immunoblotting analysis antisera were raised against FdA and FdE. Anti-FdA antiserum reacted with FdA, FdB, and FdC; anti-FdE antiserum reacted only with FdE; and FdD reacted to neither antiserum, despite its similarity to FdE in the N-terminal sequence. The strength of immunoreaction depended on antiserum-isoprotein matching. Anti-FdA antiserum exhibited its strongest reaction with FdA and a weaker reaction with FdB and FdC.

In root tissue one band was detected by immunoblotting using anti-FdE antiserum, which co-migrated with FdE. A purification of Fd from root tissue was carried out to determine its N-terminal amino acid sequence. It was then confirmed that the Fd isoprotein that was present in the root was identical to FdE of fruit (Fig. 3); only FdA and FdC were detected in leaves. During fruit growth the pigment and Fd profiles changed, as shown in Figure 4. The Chl content was maximal at the middle MG stage (27 DAA), but it was only 2.5% of that in leaf (Fig. 4A). It started to decrease dramatically just before the onset of ripening, when it was replaced by lycopene. The total abundance of Fd per gram fresh weight decreased continuously. To exclude the possibility that this decrease was mostly due to increase of water content during fruit expansion, we estimated total Fd content per microgram of protein and per gram of dry weight at each stage. In both cases Fd showed a continuous decrease during fruit development (Table I).

The content of Fd isoproteins, however, changed differentially during this period (Fig. 4B). Percentages of isoproteins to total Fd content (Fig. 4C) and content of isoproteins per microgram of protein and per gram of dry weight (Table I) were estimated to exclude the influence of water

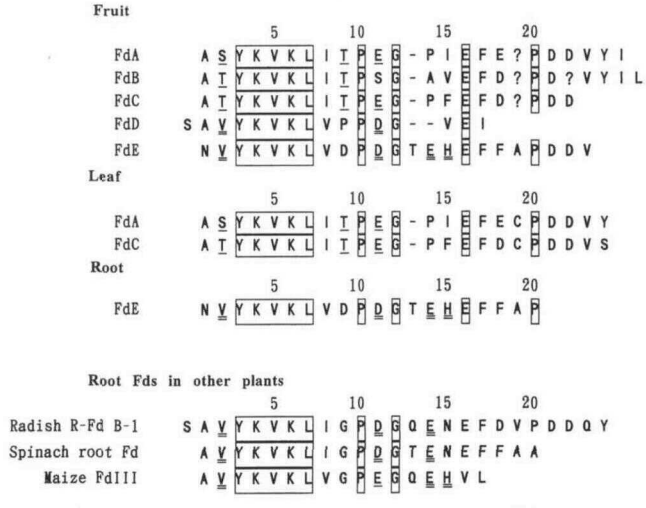

Figure 3. N-terminal amino acid sequences of tomato Fd isoproteins. ? indicates unidentified residues. Boxed letters indicate amino acids conserved in all five tomato Fd isoproteins. Underlined and double-underlined letters indicate amino acid residues found in other leaf and root Fds, respectively. Radish R-Fd B-1, spinach root Fd, and maize FdIII have been reported by Wada et al. (1989), Morigasaki et al. (1990), and Hase et al. (1991), respectively.

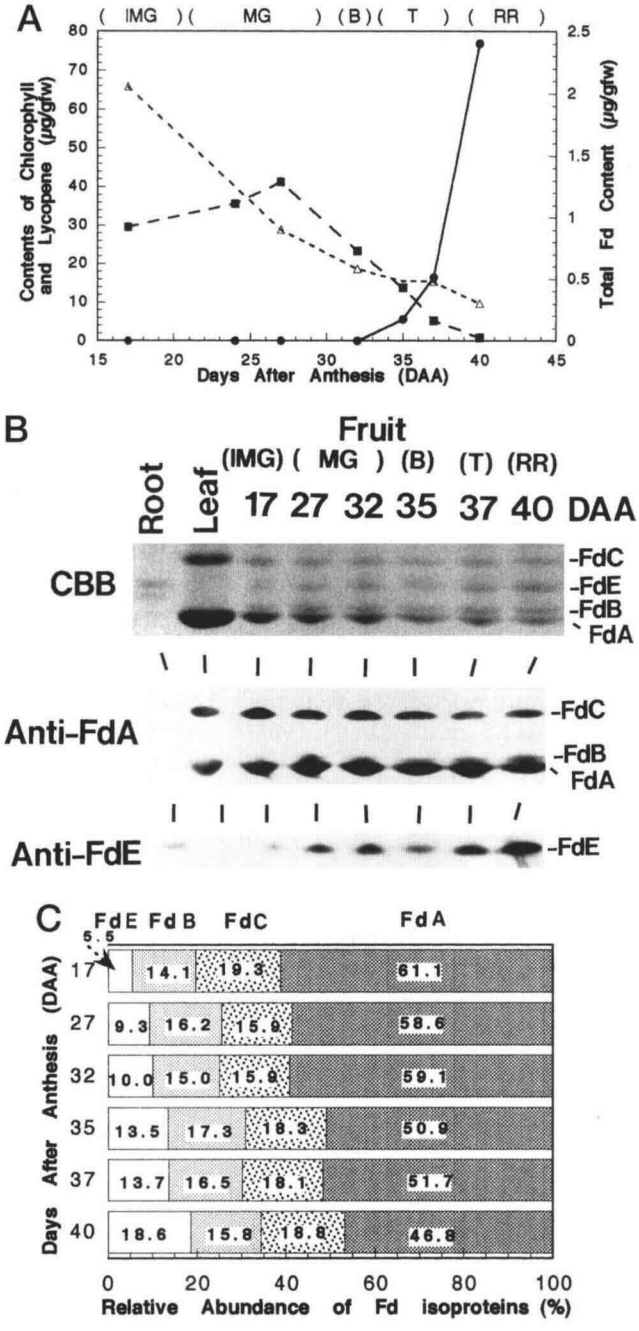

Figure 4. Temporal changes in pigment contents and accumulation of Fd isoproteins during tomato fruit development. A, Changes in pigment contents and total Fd contents. △, Total Fd; ●, lycopene; ■, Chl. Each symbol indicates a mean of three determinations. B, Coomassie brilliant blue (CBB)-staining and immunoblot analysis of Fd isoproteins. Fifty milligrams of protein was loaded onto each lane, except for the leaf lane in immunoblot analysis, which contained 20 mg of protein. For detection of immunoblotting, HyperFilm (Amersham) was exposed to chemical luminescence for 30 s. C, Temporal change of relative abundance of each Fd isoprotein. Values are percentages of each isoprotein to total Fd content calculated from band density measured by densitometry of Coomassie brilliant blue-stained gels.

content. FdA, one of the isoproteins present in leaf, decreased continuously during fruit growth, but nonetheless, was most abundant at all stages. The content of FdC, another isoprotein present in leaf, also decreased. The content of FdB was almost constant at all stages based on protein, but decreased based on dry weight (Table I). This result is inconsistent with the previous result (Kamide et al., 1995), which showed that FdB increased during ripening. It is likely that the finding of FdE led us to the different result, because the previous result was based on the relative abundance. By contrast, the percentage and content per microgram of protein of FdE increased gradually during fruit growth, especially during ripening.

All four Fd isoproteins were detected throughout fruit growth, indicating that none of the Fd isoproteins are present specifically in RR fruits.

Differential Accumulation of Fd Isoproteins in Fruit Tissues

The appearance of the cell varies significantly in different parts of the tomato fruit. The outer skin (exocarp) and the inner skin (endocarp) are made of the smallest cells. Cells in the mesocarp are the largest and are mostly vacuolated, and the columella is composed of intermediate-sized cells. In cells of parenchymatous tissues, including the mesocarp and the columella, remarkable numbers of starch granules are found, but not in the exocarp.

Considering the different appearances of cells, the content of Chl, lycopene, and starch was determined in three parts of the fruit: the exocarp, the inner pericarp (including the mesocarp and the endocarp), and the columella (Table II). In MG fruits Chl was most abundant in the inner pericarp. Chl contents in the exocarp and the columella were one-half and one-fifth of that of the inner pericarp, respectively. Lycopene was not detected in any of the tissues. Starch was more abundant in the inner part of the fruit than in the outer part. The exocarp contained only 1/30th and 1/80th levels of starch compared with the inner pericarp and the columella, respectively. In RR fruits lycopene was most abundant in the exocarp, containing 8-fold more lycopene than those in the inner pericarp and the columella. The content of starch in RR tissues was much less than that in the MG fruits.

Fd isoproteins were extracted separately from these three parts (Fig. 5). In MG fruits FdE was not detected in the exocarp. In contrast, FdE accumulated in the inner pericarp and in the columella, where abundant starch was found. The FdE/FdA ratio was higher in the columella than in the inner pericarp (Table II). In RR fruits FdE was clearly detected in the exocarp, and the FdE/FdA ratio was also higher in the inner part of the fruit (Table II). In MG fruits the FdE/FdA ratio was positively correlated to the starch-accumulation level. On the contrary, in RR fruits the FdE/FdA ratio was much higher than that in MG tissues, although only a trace amount of starch was detected. FdC was more abundant in the outer part of the fruit than in the inner part.

Table I. *Temporal changes in contents of Fd isoproteins*

Values were estimated from band densities on Coomassie brilliant blue-stained gel by using purified Fd isoproteins as standards.

DAA	Fd Content					Fd Content				
	Total	FdA	FdB	FdC	FdE	Total	FdA	FdB	FdC	FdE
	ng μg^{-1} protein					*$\mu g\ g^{-1}$ dry wt*				
17	300	183	42	58	17	25.6	15.7	3.6	4.9	1.4
27	296	174	48	47	27	13.6	8.0	2.2	2.1	1.3
32	282	167	42	45	28	8.8	5.2	1.3	1.4	0.9
35	266	135	46	49	36	7.3	3.7	1.3	1.3	1.0
37	248	128	41	45	34	7.2	3.7	1.2	1.3	1.0
40	246	115	39	46	46	6.7	3.2	1.1	1.2	1.2

Differences in Electron Transfer Activities

The electron transfer efficiencies of FdA, FdB, and FdE were investigated to obtain more insight into the relation between the differential distribution of Fd isoproteins and their functions. Apparent differences were found in the efficiency of Cyt *c* reduction activities between FdE and FdA, or between FdE and FdB. Although reduction rates did not reach the maximal level within the range tested, FdE showed a rate twice as high as FdA or FdB (Fig. 6). By regression analysis, V_{max} for FdE was estimated to be 0.052 μmol/min, which was also twice as high as the values for FdA and FdB (0.021 and 0.020 μmol/min, respectively). On the other hand, no significant difference was found between FdA and FdB. This result suggests that FdB might be functionally equivalent to FdA in terms of electron transfer, and that FdE could be more efficient in receiving electrons from NADPH via FNR than FdA and FdB.

Fd Isoproteins in Dark-Grown Fruits

The abundance of Fd isoproteins was investigated in dark-grown fruits. Under dark conditions white fruit developed and grew more slowly than their light-grown counterparts. It was confirmed by microscopic observation that the tissues in white fruit had differentiated normally, and that cell size difference was as observed in light-grown MG fruit. Whereas the Chl content was less than 5% that of light-grown fruit, dark-grown fruit accumulated as much insoluble starch as fruit grown in the light (Table II). At the last stage of development, the white fruit turned red and accumulated as much lycopene as the light-grown RR fruit.

The accumulated levels of FdA, FdB, and FdC were much less in dark- than in light-grown fruit both at MG and RR stages (Fig. 7). Dark/light ratios of FdA, FdB, and FdC at the MG stage were 30, 22, and 48%, respectively, and those at the RR stage were 30, 30, and 35%, respectively. In contrast, the dark/light ratio of FdE was more than 90% at both the MG and RR stages (Fig. 7).

These results indicated that accumulation of FdA, FdB, and FdC was controlled by the light, whereas the light seemed to have little effect on the accumulation of FdE.

DISCUSSION

Fd isoproteins of tomato can be divided into photosynthetic (FdA, FdB, and FdC) and heterotrophic types (FdE) for three reasons. First, the amino acid sequences of FdA and FdC are identical to Fd in tomato leaf, and FdB has high similarity to FdA and FdC (Fig. 3). The amino acid sequence of FdE shows high similarity to that of root Fd. Second, FdE has a Cyt *c* reduction rate twice as high as that of FdA and FdB (Fig. 6). This result

Table II. *Accumulation of pigments and starch and FdE/FdA ratio in light- and dark-grown tomato fruit tissues*

Starch was quantified as Glc. The FdE/FdA ratio is the ratio of immunoblotting band densities of FdE and FdA, and therefore does not represent the ratio of absolute amounts. Indicated values are the averages of three determinations.

Tissue	Chl		Lycopene		Starch		FdE/FdA Ratio
	Light	Dark	Light	Dark	Light	Dark	Light
	μg/g fresh wt		*μg/g fresh wt*		*μg Glc/g fresh wt*		
MG							
E[a]	14.6	1.0	N.D.[d]	N.D.	86.0	62.8	<0.05
IP[b]	30.9	0.8	N.D.	N.D.	2723.5	1804.5	0.15
C[c]	6.3	0.3	N.D.	N.D.	7147.6	9453.2	0.32
RR							
E	N.D.	N.D.	330.5	325.1	4.0	2.6	0.80
IP	N.D.	N.D.	37.7	24.3	4.5	6.4	1.23
C	N.D.	N.D.	40.4	35.4	6.1	6.3	2.03

[a] E, Exocarp. [b] IP, Inner pericarp. [c] C, Columella. [d] N.D., Not detected.

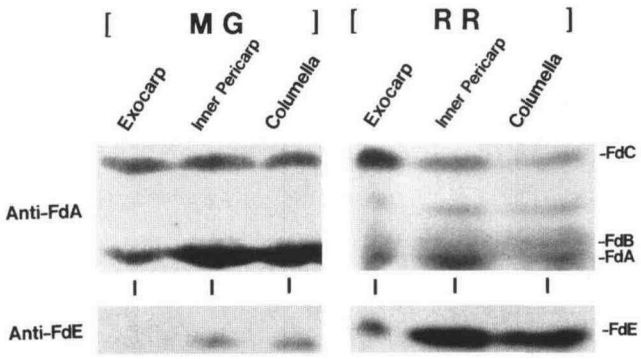

Figure 5. Immunoblot analysis of Fd isoproteins from different tissues of tomato fruit. Each lane contained 50 mg of protein, and exposure time was 30 s.

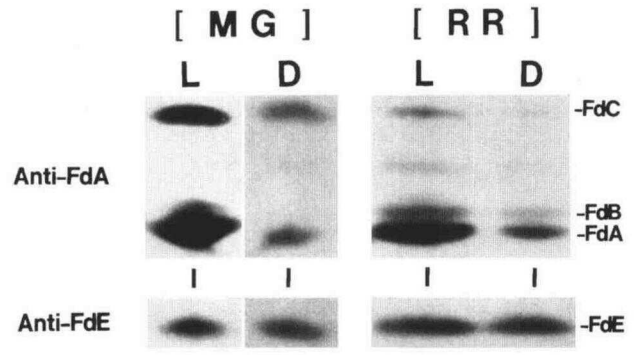

Figure 7. Immunoblot analysis of Fd isoproteins from light- (L) and dark-grown (D) tomato fruit. Each lane contained 50 mg of protein, and exposure time was 30 s.

implies that FdE could be more efficient in accepting electrons from NADPH via FNR. Such a kinetic difference between leaf and root Fd was also reported for maize (Hase et al., 1991b). Third, the accumulated level of FdA, FdB, and FdC is reduced by dark treatment, whereas the accumulated level of FdE is almost the same in the light and the dark (Fig. 7).

From a study of Fd distribution in young maize seedlings, it was suggested that plant Fds could be classified into leaf-specific and nonspecialized Fds because mRNA and mature proteins of FdI were found only in leaves, whereas those of FdIII were found in all parts of the seedling (Kimata and Hase, 1989; Hase et al., 1991a). In the present study, however, Fd isoproteins did not show leaf-specific or constitutive accumulation. Thus, it is more reasonable in the case of tomato to use the terms photosynthetic and heterotrophic type to express the differential distribution patterns of Fd isoproteins.

Only information about the N-terminal amino acid sequence is available for FdD. FdD shares identical amino acid residues at the 2nd and 11th position with FdE and root Fds of other plants (Fig. 3), and FdD is probably a heterotrophic-type isoprotein specific to the fruit. It should be noted that FdD was not found during larger-scale purification of Fds from root or leaf (data not shown).

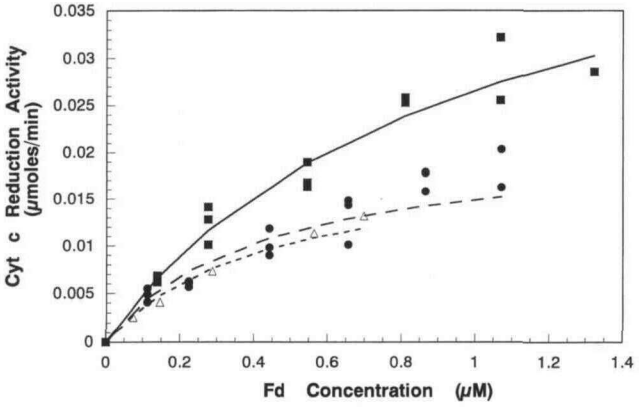

Figure 6. Cyt *c* reduction activities of FdA, FdB, and FdE. Three measurements were carried out for one Fd concentration. Fitted curves indicate results of regression analysis by using the least-squares method. ●, FdA; △, FdB; ■, FdE.

Both the photosynthetic- and heterotrophic-type Fd isoproteins are present in fruit, irrespective of photosynthetic competence. However, their temporal and spatial profiles are different. In particular, the heterotrophic-type Fd, FdE, was abundant in chromoplast-containing and starch-storing tissues (Figs. 4 and 5; Table II). The spatial profile of FdE was parallel to the distribution of mRNA of Suc synthase, which is a key enzyme of the carbohydrate transport (Wang et al., 1994). Thus, the accumulation of FdE may be related to the sink activity.

We propose a simple explanation for a physiological significance of the heterotrophic-type Fd isoprotein in fruit tissues. The photosynthetic-type Fd isoproteins decrease along with the decline of photosynthetic capability (Piechulla et al., 1987) and are less abundant in the starch-storing tissues (Table II). But reduced Fd is still required for the maintenance of anabolic and catabolic activities of the plastid, such as the assimilation of nitrogen and sulfur (Tamura, 1986) and Chl breakdown (Ginsburg et al., 1994). In the plastid with a decreased photosynthetic capability, OPP provides reducing power (Buchanan, 1980; Suzuki et al., 1985). Recently, it has been reported that fruit chloroplasts from *Capsicum annuum* L. utilized endogenous starch as a substrate for OPP (Thom and Neuhaus, 1995). It is likely that OPP of tomato fruit plastids also use starch as a source of Glc-6-P. Therefore, heterotrophic-type Fd isoprotein is required for an efficient utilization of NADPH produced by OPP in compensation for the loss of photosynthetic-type Fd isoproteins in the starch-storing and the chromoplast-containing tissues. The kinetics of Cyt *c* reduction of FdE (Fig. 6) implies that FdE is preferable to FdA or FdB for accepting electrons from NADPH, thus utilizing NADPH produced by OPP.

The distribution profile of the photosynthetic-type Fd isoproteins can be explained by the expression pattern reported so far for *fedA* of Arabidopsis (Casper and Quail, 1993), for FdI of maize (Hase et al., 1991), and for *Fed-1* of pea (Elliott et al., 1989). On the other hand, little is known about the expression of the heterotrophic-type Fd gene. Thus, we cannot conclude that the distribution profile of Fd isoproteins is a consequence of tissue-specific gene expres-

sion. Alternatively, a selective import of Fd isoproteins can explain the distribution profile. The photosynthetic-type Fd may be imported into all plastids, whereas the heterotrophic-type may be imported more efficiently into the heterotrophic plastids, such as starch-storing plastids and chromoplasts, than to the photosynthetic plastids.

In this report we considered the starch-storing tissue, together with the chromoplast-containing tissue, to be heterotrophic, and paid more attention to the difference between the photosynthetic and heterotrophic tissue than that between the chloroplast- and chromoplast-containing tissue. However, the starch-storing tissue is not merely heterotrophic because it also contains Chl (Table II). Characterization of heterotrophic plastids of MG tomato fruit is under way to elucidate Fd accumulation in more detail.

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